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Standardization of *Morinda umbellata* Linn. - An Effective Crude Drug for Diarrhoea

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Abstract: *Morinda umbellata* Linn. (Family *Rubiaceae*) is commonly known as "Nuna" in Tamil and "Pitadaru" in Sanksrit. The leaves in conjunction with certain aromatics in the form of decoction are used in diarrhoea and dysentry. The genuine sample of *M.umbellata* was collected. As part of the study genuine leaf, stem and root samples were collected and subjected to anatomical investigations. The air-dried leaf powder was extracted with different solvent systems such as petroleum-ether (40-60^{0}C), benzene, chloroform, ethanol and sterile water and preliminary phytochemical analysis of the extracts including TLC and paper chromatographic assays were done and the R_f values were determined. Physico-chemical characters, fluorescence characters and extractive values of the leaf powder in different solvent systems were also determined. The pharmacognostical parameters studied, may be used as a tool for the correct identification of the plant and also to test the adulterants if any.

Keywords: *Morinda umbellata*, Pharmacognosy, Drug standardization, Fluorescence analysis, Chromatography.

Introduction

Medicinal values of plants and herbs are immense and they are recommended for various ailments. The medicinal plant *Morinda umbellata Linn*. (Fam. *Rubiaceae*) is known as "Nuna" in Tamil and "Pitadaru" in Sanskrit. According to traditional systems of medicine, the leaf powder of *M. umbellata* is used in diarrhoea and dysentry[1-2]. The leaf powder is found to possess excellent antileukemic and antioxidant anthraquinones[3-4].

However, no pharamacognostical study has so far been reported from *M. umbellata*. This paper reports a systematic pharmacognostic study on *M. umbellata* for the first time.

Experimental

Plant material

Authentic sample of *M. umbellate* (Figure 1) was collected from Therkkumalai Estate of Courtallam Hills(altitude,350 meters), Western Ghats, Tamil Nadu, South India in the month of March 2006 using a well known taxonomist.

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Figure 1. The plant*(Morinda umbellata* Linn) showing branches and flowers

Instrumentation and techniques

A portion of fresh samples of matured leaf, stem and root of the collected plant material were fixed in FAA [Formalin: Acetic Acid: Alcohol] for anatomical studies which were carried out by following the usual plant micro technique[5]. The microphotographs were taken using the camera "PENTAX K- 1000" (Japan) fitted with the Binocular Research Microscope "LABO TRIUMPH PM – 3" and Binocular Research Microscope "HERTEL AND REUSS KASSEK" CN-hf-BIN-VK-ZT (West Germany).

The anatomical studies of the fresh leaf, stem and root were carried out using the usual plant microtechnique[5]. The details are shown in Figures 2, 3 and 4.

Figure 2.T.S. of the leaf along the midrib (x100)

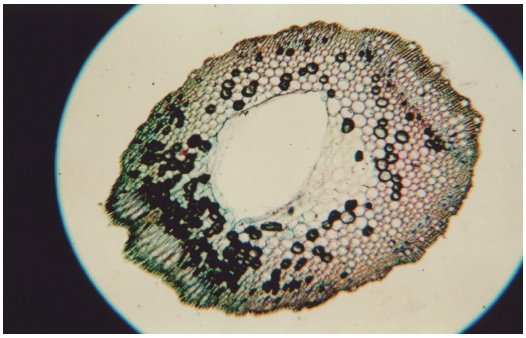

Figure 3. T.S. of stem – enlarged (x100)

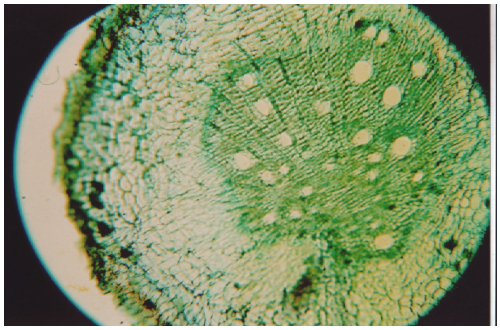

Figure 4. T.S. of root – a sector enlarged (x100)

The leaf powder of *M. umbellata* was dried under shade and powdered. The leaf powder and the extracts of the powder in various solvents were examined under ordinary light and in UV-light (365 nm). The fluorescence characters were determined according to the methods of Chase and Pratt[6]. The percentage of loss of weight on drying, total ash, water-soluble ash, acid-insoluble ash, sulphated ash and residue on ignition were determined by employing standard methods of analysis as described[7] in pharmacopoeia of India (1966). The percentage of extractive values of the leaf powder in various solvent systems was also determined.

Preliminary phytochemical analysis of the extracts of the leaf powder in various solvents has been performed[8-9]. The various extracts of the leaf powder of *M. umbellata* were subjected to thin-layer chromatographic studies using "Silicagel-G for TLC". The silicagel coated plates were dried and activated at 110^0C. UV-fluorescence lamp (365 nm) and iodine chamber were used to find out the spots and the R_f values were calculated. The water extract of the leaf powder was subjected to paper chromatographic studies using Whatmann No.1 filter paper using *n*-butanol: acetic acid: water solvent system(4.0:1.1:4.9).

Results and Discussion

Fluorescence analysis

The leaf powder of *M. umbellata* and the extracts of the powder in various solvents were examined under ordinary light and UV light (365 nm). The powder was also treated with various chemical reagents and the changes in colour were recorded. These results are presented in Table 1.

Table 1. Fluorescence characters of *Morinda umbellate* leaf powder and their extracts in different solvents.

S.No.	Particulars of treatment	*Morinda umbellata*	
		Under ordinary light	Under UV light (365nm)
1.	Powder as such	Grey	Black
2.	Powder + 1N NaOH (aqueous)	Yellowish - green	Brown
3.	Powder + 1N NaOH (ethanolic)	Yellowish - green	Brown
4.	Powder + 1N HCI	Drak green	Brown
5.	Powder + $H_2 SO_4$ (1:1)	Green	Brown
6.	Powder + HNO_3 (1:1)	Brown at the center and yellow at the edge	Dark brown
7.	**Extracts**		
	a) Petroleum ether (40^o – 60^oC)	Yellowish - green	Pinkish-orange
	b) Benzene	Brownish - green	Pinkish-orange
	c) Chloroform	Brownish - green	Pinkish-orange
	d) Ethanol	Green	Pinkish-orange
	e) Water	Dark brown	Dark-green

Quantitative determination

The percentage of loss of weight on drying, total ash, water-soluble ash, acid insoluble-ash and residue on ignition were obtained by employing standard methods of analysis as described in pharmacopoeia of India[7]. The percentage of extractive values in petroleum ether (40 – 60^0C), benzene, chloroform, ethanol and water were also determined. The results are presented in Table 2.

Table 2. Physico –chemical characters of the leaf of *Morinda umbellata*

Particulars	*Morinda umbellate*, %
Loss of weight on drying	83.20
Total ash	8.26
Acid – insoluble ash	0.71
Water-soluble ash	1.98
Sulphated ash	9.72
Residue on ignition	7.34
Extractive values	
a) Petroleum ether (40 – 60^0C)	3.52
b) Benzene	4.98
c) Chloroform	7.78
d) Ethanol	17.52
e) Water	28.16

Phytochemical screening

5g of the leaf powder of *M. umbellata* was extracted with petroleum ether (40 – 60^0C), benzene, chloroform, ethanol and water in soxhlet apparatus. The different extracts were tested for the presence of steroids, reducing sugars, carbohydrates, triterpenoids, alkaloids, phenolic compounds, saponins, xanthoprotein anthraquinones, tannins ad flavonoids. The phytochemical tests performed and the results obtained are presented in Table 3.

Table 3. Preliminary phytochemical screening of leaf powder of *Morinda umbellata*

No	Extracts	Colour	Steroids (Libermann Burchard Test)	Tri terpenoids	Reducing sugars (Tollen's test)	Carbo-hydrates (Molisch's test)	Alkaloids (Mayer's test)	Phenolic Compounds (Neutrals Ferric chioride test)	Anthra-quinones (Magnesium acetate test)	Saponins	Xantho-proteins (Nitric acid test)	Tannins (Lead acetate test)	Flavonoids (Magnesium powder test)
1.	Petroleum ether (40^0 - 60^0C)	Yellowish-green	+	-	+	+	-	-	-	-	-	-	-
2.	Benzene	Brownish - green	+	-	+	+	-	+	+	-	-	-	-
3.	Chloroform	Brownish - green	+	-	+	+	-	-	+	-	+	-	-
4.	Ethanol	Green	+	-	+	+	+	+	+	-	+	+	+
5.	Water	Pale brown	+	-	+	+	-	+	-	+	-	+	-

"+" = Presence of the compound; " –" = Absence of the compound

Thin layer chromatographic studies

Thin layer chromatographic studies have been performed for the petroleum ether (40 – 60^0C), benzene, chloroform, ethanol extracts of the leaf powder of *Morinda umbellata*. The plates were first viewed through UV-fluorescence viewing cabinet (365 nm) before keeping in an Iodine chamber and the R_f values of the fluorescing spots were noted. Then the plates were developed in the Iodine chamber and Rf values were noted of the various solvent systems tried for thin layer chromatographic studies, there was no common solvent system for all the four different extracts. Different solvent systems have been found to be effective to get the maximum number of spots for the various extracts. The results are presented in Table 4.

Paper chromatographic studies

Paper chromatographic studies have been performed for the water extracts of the leaf powder of *Morinda umbellata*. The paper first viewed through UV- fluorescence viewing cabinet (365 nm) before keeping in an Iodine chamber and the Rf values of the fluorescing spots were noted. The results are presented in Table 4.

Table 4. Thin – layer and paper chromatographic behaviour of the leaf extracts of *Morinda umbellata*

Name of the extract	Name of the solvent system used	R_f values of the spots		
		Under UV light, 365 nm	In an Iodine chamber	
Petroleum ether (40 – 60^0C)	Benzene : Chloroform (1:1)	0.05(Pink) 0.15(Pink) 0.19(Pink) 0.41(Blue) 0.98(Ivory)	0.09□ 0.21■ 0.41▧ 0.56▧	0.65□ 0.86▧ 0.98■
Benzene	Chloroform : Ethanol (9:5:0.5)	0.05(Pink) 0.72(Brown) 0.82(Pink) 0.95(Pink) 0.98(Pink)	0.08□ 0.20□ 0.28□ 0.41□ 0.52□	0.71□ 0.81□ 0.91□ 0.98■
Chloroform	Chloroform : Ethanol (9.5:0.5)	0.09(Pink) 0.29(Pink)	0.27□ 0.55□ 0.64□	0.80■ 0.91▧ 0.98■
Ethanol	Chloroform : Ethanol (8:2)	0.02(Pink) 0.14(Pink) 0.30(Pink) 0.55(Ivory) 0.23(Pink) 0.32(Pink)	0.05□ 0.15▧ 0.41□ 0.50▧	0.64▧ 0.73□ 0.98■
Water*	1 – Butanol : Acetic acid : Water, (4.0:1.1:4.9)	0.36(Pale blue) 0.88(Pale blue)	0.04□ 0.16▧ 0.23□	0.36■ 0.88■

* Paper chromatography was performed for water extract.

■-Intense ▧-Moderately Intense □-Faint

Conclusion

Thus the microscopic characters, fluorescence analysis, physico–chemical characters, preliminary phytochemical screening and thin layer and paper chromatographic studies can be used as a diagnostic tool for the correct identification of this plant. The adulterants if any in this plant material can be easily identified by using these results.

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